REMARKS

Claims 126-147 are pending; claims 132-139 are withdrawn; claims 140-143 and 145-147 are canceled; claims 126-131 and 144 are rejected; and claims 148-156 are added. Each of the rejections is addressed below.

Support for the Amendments

Support for the amendment is found throughout the application and claims as originally filed. No new matter has been added. For example, support for new claim 153, which recites "85% identity" is found, for example, at page 8, lines 1-36, at page 11, line 43, to page 12, line 10, and at Figure 1A; support for the amendment of claim 128, which now recites "the bacterial cell is present in a sample, and the method identifies a microbial infection in the sample" is found at page 21, lines 27-44; support for claim 129, which now recites that the "cell is present in a patient" is found, for example, at page 23, lines 35-40; support for new claims 148 and 154, which recite 90%, and claims 149 and 155, which recite 95% identity, and for claims 150, 151, and 156, which recite a polypeptide comprising or that is SEQ ID NO:2 is found, for example, at page 8, lines 1-36, at page 11, line 43, to page 12, line 10, and at Figure 1A; support for new claim 152 is found, for example, at pages 33-34, under the headings "Purification of RP-factor" and "Cloning of the RP-factor Gene" and at pages 37-38, under the heading "Analysis of Recombinant RP-factor."

Amendment and cancellation of the claims here are not to be construed as an acquiescence to any of the rejections/objections made in the instant Office Action or in any previous Office Action, and were done solely to expedite prosecution of the application. Applicants hereby reserve the right to pursue the claims as originally filed, or substantially similar claims in one or more subsequent patent applications.

Objection to the Specification

The Examiner's objection to the specification is overcome by the present amendment, which correctly makes reference to the Figures.

Use of Trademarks

The Examiner notes that the application includes trademarks. Applicants acknowledge that such trademarks must be capitalized, and have reviewed the specification for compliance with this requirement.

Sequence Listing

The Examiner notes that the sequence depicted in Figure 1D is inconsistent with the amino acid sequence listed in the sequence listing filed October 27, 2006. Applicants are filing herewith a revised sequence listing correcting SEQ ID NO:27.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 126-131 and 144 are rejected under 35 U.S.C. § 112, first paragraph for including new matter and for lacking enablement. For the reasons detailed below, Applicants respectfully disagree with the rejections under 35 U.S.C. § 112, first paragraph, which should be withdrawn.

New matter rejection of claims 126-131 and 144

SEQ ID NO:2 is an *M. tuberculosis* polypeptide, which is shown in Figure 1A, and which was identified as a resuscitation factor (RP-factor) based on its amino acid sequence identity to an *M. luteus* RP-factor. In Figure 1A, amino acids 117 to 184 of SEQ ID NO:2 are indicated with an asterisk, as described at page 8, lines 1-36. Applicants teach that the indicated portion of SEQ ID NO:2 includes a signaling domain that may be involved in receptor binding. In particular, at page 8, under the header "Receptor/Signaling domain class I, Applicants state, "[t]he domain may comprise a sequence of amino acid residues, the identity and relative positions of which correspond to those residues indexed by asterisks in any one of the 9 sequences set out in Figure 1A (page 8, lines 10-12)" In addition, Applicants state that "[t]he domain may also comprise derivative or equivalent sequences of amino acid residues which have at least 20% identity with any one of the particular amino acid sequences defined above, for example at least . . . 85%, 90%, 95% or 98% identity or homology therewith (page 8, lines 32-36)." Clearly, Applicants' specification provides support for independent claims 126, 144, and 153, which recite a "polypeptide comprising at least 85% identity with amino acid residues 117 to 184 of SEQ ID NO:2," claims 154 and 155, which recite 90% and 95% identity with SEQ

ID NO:2, and claims 127-131, which depend from claim 126. Thus, the new matter rejection of claims 126-131 and 144 should be withdrawn. Furthermore, the new matter rejection should not apply to claims 148-156.

Enablement rejection of claims 126-131 and 144

At page 6, paragraph 9, of the Office action mailed on February 1, 2007, the Examiner acknowledges that Applicants have enabled methods for resuscitating dormant cells of *Micrococcus luteus* cells, *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, and *Mycobacterium bovis* using an RP-factor of *M. luteus*. However, the Examiner asserts that Applicants have failed to enable a polypeptide having 20% to 50% identity or homology with residues 117-184 of SEQ ID NO:2. Applicants respectfully disagree and traverse the rejection.

The number of living cells in a bacterial culture is typically assayed by measuring the ability of the cells to grow and divide on an agar bacterial culture plate (page 2, lines 5-6). Certain bacterial cells may exist in a "dormant" "latent" or "moribund" state, where they cannot be cultured on agar plates under standard growth conditions (page 2, lines 5-9). Such cells are not dead, however, because they can be resuscitated (i.e., induced to grow in culture) (page 2, lines 10-11). The existence of "latent" pathogenic bacteria has important implications for human health related to bacterial infection (page 2, lines 23-26). The pathogenic bacteria, *M. tuberculosis*, for example, persists for long periods of time in a "latent" state that is difficult to detect in standard diagnostic methods (page 2, lines 16-26).

The Examiner alleges that Applicants have failed to provide guidance regarding where amino acid substitutions may be made in the polypeptide. Applicants respectfully disagree. Using sequence information relating to *M. luteus* RP-factor, Applicants have identified RP factor proteins from other bacteria, including SEQ ID NO:2 from *M. tuberculosis*, that share sequence identity with *M. luteus* RP-factor (page 34, line 21, to page 35, line 3, under the header "Identification of RP-factor homologues"), and Applicants have used this information to identify conserved structural features. Specifically, Applicants have identified two RP-factors from *M. luteus* and one from *M. tuberculosis* (Figure 1A; page 34, line 21, to page 35, line 4). In addition, Applicants have identified RP-factors from *M. leprae* and *Streptomyces coelicolor*, *Streptomyces rimosus, Mycobacterium smegmatis,* which includes four similar genes,

Mycobacterium bovis, and *Cornebacterium glutamicum,* which includes two similar genes (Figure 1A; page 34, line 21, to page 35, line 4). Applicants have provided an alignment of RP factor proteins in Figure 1A, which identifies conserved structural features and highly conserved amino acid residues (page 35, lines 5-34; Figures 9A and 9B). Applicants found that RP-factors share a secretory signal sequence and a conserved 70-residue segment that may act as a signaling domain (page 35, lines 5-18, under the heading "Domain structure"). This domain includes four conserved tryptophan residues and two conserved cysteine residues that may form a disulfide bridge (page 35, lines 25-30). These structural features are conserved among a wide variety of proteins and are, therefore, likely to be functionally important. Accordingly, Applicants' specification provides guidance relating to those regions of the protein where sequence variations are likely to be tolerated and those conserved regions where variations in the sequence are less desirable.

Moreover, one of skill in the art could readily identify those variant polypeptides that fall within the scope of Applicants' claims (i.e., those polypeptides having at least 20%, 50%, 85%, 90%, or 95% amino acid sequence identity to SEQ ID NO:2 that are capable of resuscitating dormant bacteria) using routine methods that are clearly described in Applicants' specification. For example, Applicants' specification clearly describes methods of screening for polypeptides capable of resuscitating dormant bacteria using purified RP-factors (page 33, line 20, to page 34, line 3, page 35, lines 35-44). Such screening does not constitute undue experimentation because it could easily be accomplished using standard techniques that are plainly described in Applicants' specification. In analyzing what constitutes undue experimentation, the MPEP (§ 2164.06) cites *In re Wands*, (858 F.2d 731, 8 USPQ2d 1400 (Fed Cir. 1988)):

> The test is not merely quantitative, since ***a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance*** with respect to the direction in which the experimentation should proceed. (emphasis added)

The present situation is, in all important aspects, indistinguishable from the facts in Wands in which the Federal Circuit held that the applicant's claim was enabled, despite the necessity for screening, because the process of screening was straightforward. Thus, using no more than routine methods, the skilled artisan could readily identify those polypeptides having at least 20% identity to SEQ ID NO:2 that are capable of resuscitating dormant bacteria.

The Examiner alleges that "the function of SEQ ID NO:2 is unknown" and the biological activity of a portion of SEQ ID NO:2 containing amino acids 117-184 is also unknown (Office action mailed February 1, 2007, page 10, lines 1-15). Applicants respectfully disagree. Applicants have plainly disclosed a biological activity for SEQ ID NO:2, including that portion of the polypeptide containing amino acids 117-184 (page 35, lines 35-44). Applicants expressed a secreted form of the *M. tuberculosis* polypeptide in *E. coli* (page 38, line 31 to page 39, line 10). This fragment of SEQ ID NO:2 included amino acids beginning at D50 of the amino acid sequence, and included amino acids 117-184 as recited in the claims (page 39, lines 11-15). The purified protein was added to cultures of *M. luteus* and *M. tuberculosis*. Applicants found that as expected SEQ ID NO:2 stimulated the growth of *M. tuberculosis* cells and *M. luteus* cells (page 39, lines 24-34, under the header "Effect of *M. luteus* RP-factor on growth of Mycobacterium tuberculosis cells isolated from macrophages"). This growth stimulation occurred under conditions where a control culture had ceased to grow (page 39, lines 19-22). Applicants found that the control culture grew to a final OD_{600nm} of 1.0 (page 39, lines 19-22). In contrast, cultures treated with purified RP-factor continued to grow to final OD_{600nm} of 2.0-6.0 (page 39, lines 20-22). These results indicated that a SEQ ID NO:2 polypeptide containing amino acids 117-184 stimulated cell proliferation under conditions where the control culture failed to grow, just as the *M. luteus* RP-factor did (page 35, line 35, to page 36, line 7). In view of this disclosure, Applicants have clearly described a biological activity for a SEQ ID NO:2 polypeptide containing amino acids 117 to 184 of SEQ ID NO:2.

In support of the present claims, Applicants provide herewith additional data in Exhibit A (Figures 1-4) showing that polypeptides having about 20% identity to *M. luteus* RP-factor have a biological activity associated with *M. luteus* RP-factor. Recent work has shown that RP-factor proteins are murein hydrolases that have cell wall lytic activity. Cell wall lytic activity is likely to be important for resuscitating dormant, moribund or latent bacterial cells. The walls of dormant bacterial cells contain an excess of an inert peptidoglycan. This excess peptidoglycan restrains the growth of the bacteria. The cell wall lytic enzyme activity is required to make a restricted number of scissions in the wall, thereby allowing bacterial cell growth and wall expansion to occur. Figures 1 and 2 provide sequence alignments of proteins having about 20% identity to RP-factor. Figure 1 shows sequence alignment of the Rpf-like domain of two *T. whipplei* enzymes, and a number of gene products from related organisms with an Rpf domain

consensus sequence. The Rpf domain consensus sequence is based on over 40 individual Rpf sequences. Figure 2 shows a sequence alignment of the Rpf-like domain of a *T.whipplei* enzyme, TW325, with other Rpf domain sequences. The percent identity and homology (similarity) values for each of the Rpf domain sequences with TW325 are shown in Figure 2. The % homology between TW325 and the Rpf domain sequences ranges between 20% and 28.8%. Applicants note that sequences having 20% to 50% identity to SEQ ID NO:2 fall within the scope of the claims. Figure 3 shows a gel (zymogram) that shows that TW325 has murein hydrolytic activity. In addition, Applicants have shown that a suspension of *M. luteus* cell wall fragments loses up to 50% optical density following incubation with recombinant Rpf, providing further evidence that the Rpf has cell wall lytic activity. Finally, Applicants have shown that diaminopimelic acid-containing material is released into the soluble fraction using fluorescent-labeled cell walls. The experiments described in Figure 4 show that both Rpf proteins and TW325 have murein hydrolytic/cell wall lytic activity. Bacteria may become dormant due to a reduction of nascent peptidoglycan in the bacterial cell wall and its gradual replacement by inert peptidoglycan. Cleavage of the cell wall likely provides for resumption of cell wall synthesis and the subsequent re-initiation of protein synthesis, which is important for the resuscitation of dormant, moribund or latent bacterial cells.

In summary, Exhibit A shows that TW325, which is 20 and 28.8% homology to Rpf domain sequences, possesses the same biological activity (i.e., murein hydrolase activity) as other RP-factor proteins. Such activity is related to the resuscitation of dormant, moribund or latent bacterial cells. In view of this evidence, other polypeptides having at least about 20% identity to SEQ ID NO:2 would also be expected to have murein hydrolase activity and to function in the resuscitation of dormant, moribund or latent bacterial cells. Accordingly, the enablement rejection of claims 126-131 and 144 should be withdrawn.

In support of the enablement rejection, as it applies to claims 128-131, the Examiner alleges that Applicants have failed to enable the use of polypeptide variants of SEQ ID NO:2 in therapy, prophylaxis, or diagnosis. Applicants disagree and traverse the rejection. Nevertheless, the claims are now directed to methods for identifying a microbial infection in a sample (claim 128) and to methods for resuscitating a bacterial cell where the cell is present in a patient being treated with an antimicrobial. Applicants have clearly shown that RP-factors may be used to

resuscitate bacterial cells, including dormant *M. tuberculosis* cells isolated from a mouse infected with *M. tuberculosis* (page 39, line 24, to page 40, line 27). At Table 2, in Experiment III, Applicants showed that an RP-factor was able to stimulate the growth of *M. tuberculosis* cells that failed to show signs of viability (page 39, Table 2). In view of this disclosure, Applicants have clearly enabled methods of resuscitating bacterial cells in a sample or a patient. Thus, this basis for the enablement rejection should also be withdrawn.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 126-131 and 144 are rejected for indefiniteness. Applicants respectfully disagree and traverse the rejection.

The Examiner rejected claims 126 and 144 for failing to specify that SEQ ID NO: 2 provides an amino acid sequence. The claims recite "amino acid residues 117 to 184 of SEQ ID NO:2." In view of this language, one skilled in the art would appreciate that SEQ ID NO:2 represents an amino acid sequence. Thus, this basis for the indefiniteness rejection should be withdrawn.

The rejection of claims 126 and 144 is rendered moot by the present amendment, which deletes the terms "homologue," "species variant," and "mutein." Accordingly, this basis for the indefiniteness rejection should be withdrawn.

The rejection of claim 128 is overcome by the present amendment. Claim 128 now recites that the bacterial cell is present in a sample, and the method diagnoses a microbial infection in the sample. Accordingly, this basis for the indefiniteness rejection should be withdrawn.

The rejection of claim 129 is also overcome by the present amendment. Claim 129 now recites that the bacteria is present in a patient being treated with an antimicrobial.

Applicants believe that the rejection of claim 129, for reciting a "strain expressing a nucleic acid encoding" was made in error. Applicants note that claim 129 does not include the aforementioned phrase. The rejection is addressed with respect to claim 144, which recites "a cell strain expressing a nucleic acid encoding a polypeptide." The Examiner alleges that it is

unclear whether the cells are expressing a nucleic acid or a polypeptide. Applicants respectfully disagree. One of skill in the art will appreciate that where cells express a particular polypeptide, the cells necessarily express the nucleic acid sequence encoding the polypeptide. Thus, the claim is definite as written. Accordingly, this basis for the indefiniteness rejection should also be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 126, 127, 130, 131, and 144 are rejected under 35 U.S.C. § 102(a) as anticipated by Mukamolova et al., (Arch. Microbiol. 172:9-14, 1999), as evidenced by Mukamolova et al., (PNAS 95:8916-8921, 1998). The Examiner alleges that in view of the rejection of the claims under 35 U.S.C. § 112, first paragraph, for including new matter, Applicants' "instant claims are granted the filing date of the instant application, i.e., 5/11/00 (Office action mailed February 1, 2007, page 15, paragraph 13)." Applicants respectfully disagree and traverse the rejection.

As detailed above, Applicants' specification as originally filed clearly provides support for the claimed invention. Specifically, support for claims 126 and 144, which recite "amino acids 117 to 184 of SEQ ID NO:2" is found at Figure 1A; support for claims 126 and 144, which recite "85% identity" to SEQ ID NO:2 is found at page 8, lines 1-36, at page 11, line 43, to page 12, line 10. The present application is the U.S. national phase of PCT/GB98/01619, which was filed on June 3, 1998, and claims the benefit of UK 9811389.8 and UK 9811221.2, which were filed on June 4, 1997 and May 27, 1998, respectively. Mukamolova et al., (Arch. Microbiol. 172:9-14, 1999) and Mukamolova et al., (PNAS 95:8916-8921, 1998) were published after Applicants' priority date, and are, therefore, not available as prior art. Thus, the rejection of the claims under 35 U.S.C. § 102(b) should be withdrawn.

Claims 126, 127, 130, 131, and 144 are further rejected as anticipated by Mukamolova et al., (Antonie van Leeuwenhoek 67:289-295, 1995; hereinafter "Mukamolova 1995"). The Examiner alleges that "every element of the claimed subject matter is disclosed by Mukamolova et al. (1995) with the unrecited characteristics being inherent therein." For the reasons discussed below, Applicants respectfully disagree with the rejection and request that it be withdrawn.

To serve as an anticipation, a cited reference must describe all of the elements of the claimed invention. In *Verdegaal Bros. V. Union Oil Co. of California*, 814 F.2d 628, 631, 2

USPQ2d 1051, 1053 (Fed. Cir. 1987), the Federal Circuit held that "a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Mukamolova fails to meet this standard.

Mukamolova 1995 describes the use of a cultural supernatant from *Micrococcus luteus* bacterial cells to resuscitate dormant bacterial cells. In contrast, Applicants' claims are directed to the use of an isolated polypeptide having at least 20%, 50%, 85%, 90%, or 95% identity SEQ ID NO:2, an RF-factor polypeptide of *M. tuberculosis*, to resuscitate dormant bacterial cells. Mukamolova 1995 fails to describe the use of any isolated polypeptide or the use of any *M. tuberculosis* polypeptide. Thus, Mukamolova 1995 fails to anticipate the claimed invention. Accordingly, the rejection under 35 U.S.C. § 102 of claims 126, 127, 130, 131, and 144 should also be withdrawn.

CONCLUSION

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

Applicants believe that no fee is due to consider the present amendment. Nevertheless, the Director is hereby authorized to charge or credit any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105.

Dated: August 1, 2007

Respectfully submitted,

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